

Fosfomycin: mechanisms and the increasing prevalence of resistance

Mina Aghamali,¹ Mansour Sedighi,² Abed Zahedi bialvaei,² Nima Mohammadzadeh,² Sara Abbasian,² Zahra Ghafouri³ and Ebrahim Kouhsari^{2,*}

Abstract

There are challenges regarding increased global rates of microbial resistance and the emergence of new mechanisms that result in microorganisms becoming resistant to antimicrobial drugs. Fosfomycin is a broad-spectrum bactericidal antibiotic effective against Gram-negative and certain Gram-positive bacteria, such as Staphylococci, that interfere with cell wall synthesis. During the last 40 years, fosfomycin has been evaluated in a wide range of applications and fields. Although numerous studies have been done in this area, there remains limited information regarding the prevalence of resistance. Therefore, in this review, we focus on the available data concerning the mechanisms and increasing resistance regarding fosfomycin.

INTRODUCTION

The alarming increase in antibiotic resistance rates reported among various pathogens has resulted in the employment of alternative treatment policies. Since the availability of novel antimicrobial drugs is rather limited, the reassessment of older antibiotic agents appears to be an interesting option [1, 2]. Fosfomycin (also known as phosphomycin), a bactericidal phosphoenolpyruvate (PEP) analogue previously used as oral treatment for uncomplicated urinary tract infections (UTIs), has recently interested clinicians all around the world [3]. Mostly, the reported advanced resistance against pathogens suggests that fosfomycin can serve as an appropriate treatment option in patients with highly resistant microbial infections. Although many researches have been carried out on fosfomycin's characterization, there remains no centralized information regarding the current level of resistance. In this review, referring to the key data available, we focus on the those concerning the prevalence of fosfomycin resistance.

DEVELOPMENT, CLASSIFICATION AND BIOCHEMICAL CHARACTERIZATION

The history of fosfomycin began in 1969, when it was first isolated from *Streptomyces fradiae* in soil samples using broth cultures [4, 5]. It was subsequently extracted from *Streptomyces viridochromogens* and *Streptomyces wedmorensis*, by American and Spanish researchers, independently [in a

joint effort of Merck and Co. and Spain's Compañía Española de Penicilina y Antibióticos (CEPA)] [4, 6]. However, this antibiotic was soon developed by chemical synthesis and its medical use began in 1971 [7]. Fosfomycin was approved by the USA in 1997 and commonly used for uncomplicated UTI caused by susceptible organisms [8–10]. Phosphonic acid derivatives, and particularly fosfomycin, attracted many researchers: its unique mechanism and structure are at the core of its clinical properties and particular actions [11–13]. Despite fosfomycin's efficacy and favourable characteristics some problems arose, including the poor bioavailability of the drug when administered by the oral route, the variation in activity in different culture media and, consequently, difficulties in determination of its antimicrobial activity and the rapid emergence of resistant strains [14, 15].

Fosfomycin has the smallest molecular mass among existing antibiotics (138 Da), which confirms its wide ability to diffuse. It is a strongly polar molecule, soluble in water and its pharmacological properties are unchanged under normal storage conditions for 2–3 years. Fosfomycin is unstable in an acidic environment and thus has poor oral bioavailability in the disodium salt form, which is used for the parenteral route [8]. However, fosfomycin-trometamol is a hydro-soluble product, facilitating use of the oral route of administration [16].

Received 30 January 2018; Accepted 23 October 2018; Published 15 November 2018

Author affiliations: ¹Department of Microbiology, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran; ²Department of Microbiology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran; ³Department of Biochemistry, Biophysics and Genetics, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran.

***Correspondence:** Ebrahim Kouhsari, ekouhsari1987@gmail.com

Keywords: Fosfomycin; Fosfomycin-Trometamol; urinary tract infections; resistance; antibiotic.

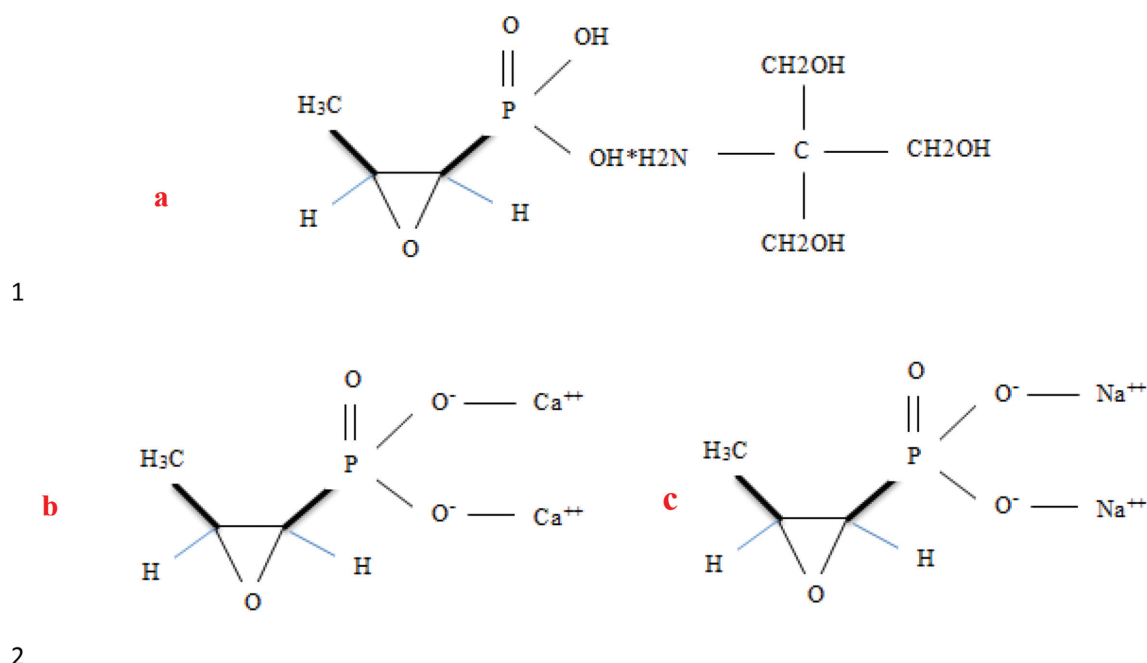


Fig. 1. (a) Molecular structure of fosfomycin-trometamol. (b) Molecular structure of fosfomycin-calcium. (c) Molecular structure of fosfomycin-disodium.

Fosfomycin is associated with phosphonic antibiotics, of which there are three types administered by the parenteral route: fosfomycin, fosmidomycin and alafosfalin. Primarily, fosfomycin disodium is administered parenterally to patients with serious infections, such as meningitis [9]. Recently it has been produced in an oral form, fosfomycin-trometamol, a monobasic hydro-soluble fosfomycin salt administered specifically in UTIs. Fosfomycin-trometamol is accessible in the form of a white crystalline powder. With the exception of fosfomycin-trometamol salt, the available data on other related products is limited [8]. In certain European countries, fosfomycin disodium is sometimes used to treat patients with soft tissue infections and sepsis [17].

In many countries, including Spain, France, Austria, Brazil, Germany, Japan and South Africa, fosfomycin has been used effectively via the intravenous route for nearly four decades [17]. In Europe, the utilization of intravenous fosfomycin disodium for patients with sepsis, soft tissue infections and deep-seated infections has become well established over the last 18 years. Conversely, the United States Food and Drug Administration (FDA) has affirmed the use of oral fosfomycin-tromethamine alone for the treatment of uncomplicated lower UTIs caused by *Escherichia coli* or *Enterococcus faecalis*, due to limited clinical analysis [17]. Unfavourable results, predominantly in regard to the treatment of acute gonococcal urethritis, have led to a limited acceptance of fosfomycin in the United States. However, 40 years of clinical experience documents that intravenous fosfomycin is effective and well tolerated in a

variety of patient populations. Consequently, fosfomycin could be an appropriate choice in the therapy of deep-seated and severe infections [18]

Chemical structure

Fosfomycin ([[(2R, 3S)-3-methyloxiran-2-yl] phosphonic acid), is an exclusive antibiotic whose chemical structure is unlike that of other known antibacterial agents [19]. Being small and hydrophilic, it shows insignificant serum protein-binding tendency [20]. The molecular structure of fosfomycin varies based on the available drug formulations. Generally, it is accessible in two oral formulations, fosfomycin-trometamol (or fosfomycin-tromethamine) ($C_3H_7O_4P \cdot C_4H_{11}NO_3$) (Fig. 1a) and fosfomycin-calcium ($C_3H_5CaO_4P$) (Fig. 1b). The intravenous formulation of fosfomycin is fosfomycin disodium ($C_3H_5Na_2O_4P$) (Fig. 1c) [21].

PHARMACOKINETIC/PHARMACODYNAMIC PROPERTIES

As antibiotic selection options gradually decrease, fosfomycin assumes increasing importance due to its efficacy against multidrug-resistant pathogens [22, 23]. First is its oral bio-availability that allows it to actively reach tissues and maintain high blood levels, and the achievement of therapeutic results before resistance occurs. Fosfomycin-trometamol appears to fit this description [24]. In fact, fosfomycin-trometamol was shown to have, in comparison to other phosphonic acid derivatives, (1) a similar safety profile [25]; (2) a similar profile of bacteriological activity

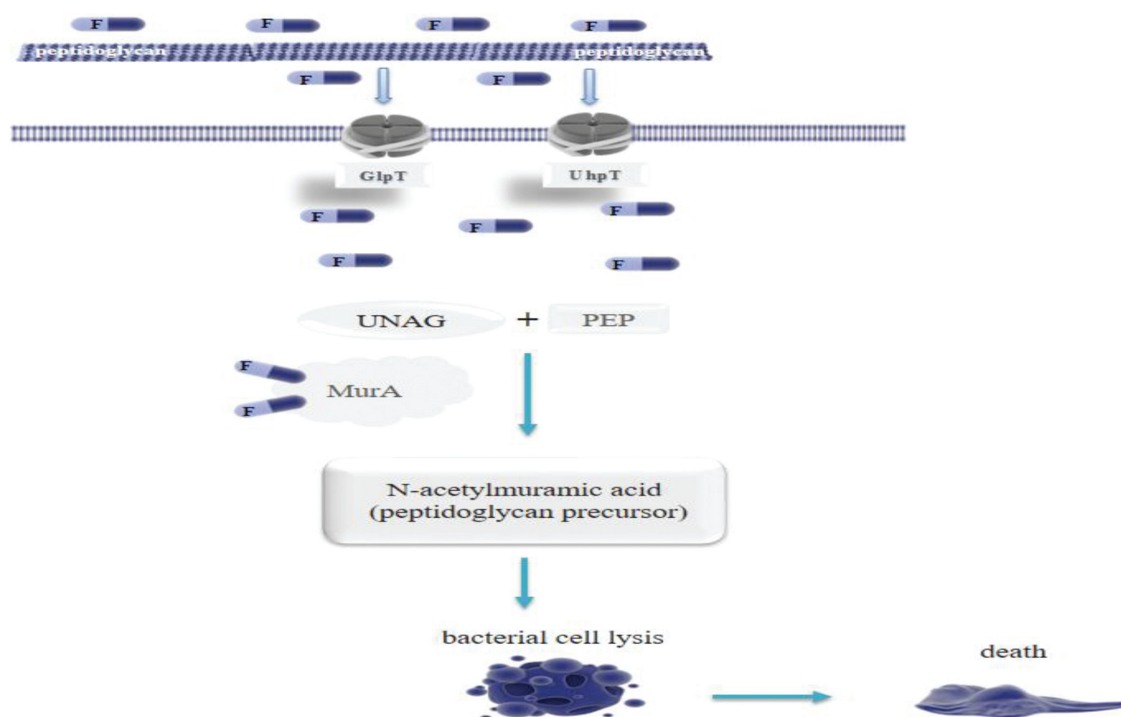


Fig. 2. Mechanism of action of fosfomycin.

comparable to other agents [26, 27]; and (3) enhanced oral bioavailability [28, 29].

Fosfomycin is rapidly absorbed by the oral route, and its bioavailability is almost 40 % for fosfomycin-trometamol vs. 12 % for the fosfomycin-calcium salt. Fosfomycin has good tissue dissemination in different sites such as the lungs, serum, prostate, kidneys, liver, cerebrospinal fluid, pus, bladder wall, inflammatory tissue, heart valves and bone [30, 31]. It is excreted in the urine unchanged, reaching high concentrations over a period of time [9]. About 30–60 % of fosfomycin-trometamol is excreted unchanged via the urine, versus 9–18 % for the calcium salt [28]. Fosfomycin has a renal exclusion of 95 %, with no tubular secretion, and a relatively long elimination half-life, ranging 4–10 h [32]. Due to the low rate of fosfomycin recovery in patients with chronic renal failure, its half-life is enhanced significantly (up to 50 h) [32]. The serum concentration of fosfomycin is higher when administered prior to the intake of food, pharmacokinetic parameters showing a significantly decreased absorption of the compound. Fifty-one per cent of the dose consumed is excreted in the urine over 24 h [33, 34]. Urinary fosfomycin concentrations are high and may exceed 2000 mg l^{-1} after a single dose, they remain high for a long time (usually more than 24 h), resulting in a challenge regarding common therapies for UTIs [8].

When considering fosfomycin MIC_{50} and MIC_{90} concentrations against pathogenic bacteria, it is clearly understood that this agent is especially active against the most common

pathogens causing UTIs, including *E. coli*, *Enterobacter* spp., *Citrobacter* spp. and *Proteus mirabilis*. However, its susceptibility rate can vary among these pathogens. However, on the basis of pharmacokinetic behaviour, the bioavailability of fosfomycin is far better than that of its calcium salt [24]. After oral administration of the calcium or trometamol salt (50 mg kg^{-1}), the peak serum level of the former was almost 30 mg l^{-1} while that of the latter is near 5 mg l^{-1} . Almost 10 h after administration of fosfomycin-trometamol, the serum level of fosfomycin was $3\text{--}5 \text{ mg l}^{-1}$ [35]. These results show an extended period of time in the urine – an unexpected finding – since the half-life of fosfomycin-trometamol is about 3–4 h, which has been attributed to the enterohepatic recirculation of the drug as recognized by a secondary peak in the serum level curve [16, 36]. These observations in the preliminary phase of fosfomycin-trometamol studies led to the conclusion that fosfomycin-trometamol is a very safe drug, as well as the recognition that it delivers high bactericidal concentrations of the drug to the urine for at least 36–48 h. Therefore, researchers have now reconsidered this drug. Indeed, the high urinary levels facilitate long-lasting bactericidal activity and inhibit the presence of resistant strains. These characteristics are required for a drug specifically indicated for ‘single-dose treatment’ of lower uncomplicated UTIs [37, 38]. The ideal drug profile for single-dose therapy of lower UTIs includes the following: (1) Very low toxicity; (2) bactericidal activity against most uropathogens; (3) high drug concentrations in the urine over an extended period; (4) no emergence of resistance; and (5) no cross-

resistance with other antibiotics. These parameters are achieved by fosfomycin-trometamol, providing the background for the clinical acceptance of fosfomycin-trometamol in lower uncomplicated UTIs [1, 39, 40].

The pharmacodynamic index that best links drug exposure to antimicrobial efficacy is significant for understanding the optimal use of fosfomycin, in terms of bacterial killing and/or preventing the development of resistant populations. Historically, fosfomycin has been considered as an agent with a time-dependent antimicrobial effect [20]. In a study by Docobo-Pérez, dose fractionation research was conducted with two ESBL-producing strains with a fosfomycin MIC of 1 mg l^{-1} . In both cases, the administration of fosfomycin resulted in the same level and extent of bacterial killing, irrespective of the administration schedule [20]. In another study, the pharmacokinetics of fosfomycin were surveyed in mice infected with Gram-negative bacterial pathogens (*E. coli*, *K. pneumoniae* and *P. aeruginosa*) after subcutaneous administration of 3.125, 12.5, 50, 200, 400 and 800 mg kg^{-1} . The half-life ranged from 0.51 to 1.1 h, the area under the concentration–time curve ($\text{AUC}_{0-\infty}$) ranged from 1.4 to $87 \text{ mg} \times \text{h l}^{-1}$, and maximum concentrations ranged from 0.6 to 42.4 mg l^{-1} . Dose fractionation demonstrated the AUC/MIC ratio to be the PK/PD index, most closely linked to efficacy ($R^2=0.70$). Net stasis and bactericidal activity were detected against all isolates [41]. These findings should prove useful in the design of clinical dosing regimens for fosfomycin in serious infections due to *Enterobacteriaceae* and *Pseudomonas*.

MODE OF ACTION

Fosfomycin has a bactericidal action that inhibits the biosynthesis of peptidoglycan in both Gram-positive and -negative bacteria during the first step, leading to bacterial cell lysis and death [13]. Fosfomycin acts as a phosphoenolpyruvate (PEP) analogue and binds to MurA (UDP-GlcNAc enolpyruvyl transferase), an essential enzyme for peptidoglycan biosynthesis [42], catalysing the transfer of the enolpyruvyl moiety of PEP to the 3'-hydroxyl group of UDP-N-acetylglucosamine (UNAG) [43]. Thereby, it prevents the formation of N-acetylmuramic acid from N-acetyl-glucosamine and phosphoenolpyruvate, finally resulting in bacterial cell lysis and death (Fig. 2) [44, 45]. In fact, the effect of fosfomycin on the active site of MurA inhibits this enzyme by a covalent thioether bond formation with a key residue in the active site, Cys115 [44]. Fosfomycin uses the glycerol-3-phosphate transport (GlpT) and hexose phosphate uptake transport (UhpT) systems (providing an alternative for GlpT system for its influx into cells) as methods of entry in almost all susceptible bacteria to achieve membrane lysis of the target pathogen (Fig. 2) [45–47].

International committees for susceptibility breakpoints

The only approved minimum inhibitory concentration (MICs) method for testing is agar dilution, using agar media

supplemented with $25 \text{ } \mu\text{g ml}^{-1}$ glucose-6-phosphate. Broth dilution MIC testing should not be performed. Breakpoints to define resistance for licensed use differ: $\geq 256 \text{ mg l}^{-1}$ according to the Clinical and Laboratory Standards Institute (CLSI), $>128 \text{ mg l}^{-1}$ according to the British Society for Antimicrobial Chemotherapy and $>32 \text{ mg l}^{-1}$ according to the Committee on Antibiograms of the French Society of Microbiology. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) has defined the clinical MIC breakpoint for fosfomycin and enterobacteriaceae as $R>32 \text{ mg l}^{-1}$ [48, 49]. Specifically, according to the CLSI, MIC values should be interpreted on the basis of the following criteria: $\text{MIC} \leq 64 \text{ mg ml}^{-1}$: susceptible (S); $\text{MIC } 128 \text{ mg ml}^{-1}$: intermediate (I); $\text{MIC} \geq 256 \text{ mg ml}^{-1}$: resistant (R) [21].

Activity against Gram-negative bacteria

Studies have revealed a stable susceptibility over time against common uropathogenic Gram-negative bacteria, including *E. coli*, *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* [50, 51]. Although *P. aeruginosa* and *Stenotrophomonas maltophilia* showed moderate susceptibility, *A. baumannii* isolates were intrinsically resistant to fosfomycin monotherapy [44, 52]. However, a combination of fosfomycin with other antibiotics (cefepime, aztreonam or meropenem) was effective in an *in vitro* study involving *P. aeruginosa* clinical isolates [53]. Fosfomycin is not active against anaerobic bacteria such as *Bacteroides* spp., but it is active against *Peptococcus* and *Peptostreptococcus* spp. [54].

Activity against Gram-positive bacteria

Fosfomycin is also very effective against Gram-positive cocci, including *Staphylococcus aureus*, *Enterococcus faecalis*, *Staphylococcus epidermidis*, *Enterococcus faecium* and *Streptococcus pneumoniae* [6]. It has *in vitro* effective activity against *S. aureus* (MIC_{50} , 8 mg l^{-1} ; MIC_{90} , 16 mg l^{-1}) [55]. *Listeria monocytogenes* is resistant to fosfomycin, whereas other *Listeria* species (e.g. *Listeria ivanovii*) may be susceptible [56]. However, intrinsically resistant bacteria to fosfomycin include *Staphylococcus capitis*, *Staphylococcus saprophyticus* and *Mycobacterium tuberculosis* [54].

Previous studies have thus indicated that fosfomycin is a successful treatment option for infections caused by multi-drug-resistant (MDR) Gram-positive and -negative pathogens [39, 57]. However, fosfomycin resistance needs to be continuously surveyed and its molecular characteristics have to be understood to prevent the future emergence of and increase in MDR bacterial strains with fosfomycin resistance in the clinical setting.

MECHANISMS OF RESISTANCE

Bacterial resistance to fosfomycin can be either chromosomal or plasmid mediated. Most chromosomally resistant mutants that do not easily transfer to other organisms have an impaired uptake system [58], whereas plasmid-resistant mutants are generally known to be multi-resistant and can transfer their resistance to other organisms through

conjugation or transformation [58, 59]. Bacterial resistance to fosfomycin occurs via three mechanisms, two of which are based on chromosomal genes and the third on plasmids [60].

Reduced permeability to fosfomycin

The main mechanism for acquisition of fosfomycin resistance is an inactivation in phosphonate transport or uptake pathways [61]. In *E. coli*, two main nutrient transport systems, the glycerol-3-phosphate transporter (GlpT) and the glucose-6-phosphatetransporter (UhpT), are responsible for fosfomycin uptake [62]. Expression of these genes (*glpT*, *uhpT*) requires the presence of cAMP levels that can be lowered by mutations in the *ptsI* or *cyaA* genes (which will also affect catabolism in a variety of carbohydrates), and for the *uhpT* gene, high-level expression requires the regulatory gene *uhpA* [62–64]. Mutations in each regulatory gene of those pathways decrease antibiotic uptake, conferring different levels of fosfomycin resistance [62, 65].

Strains defective in fosfomycin uptake are not able to grow using only a carbon source, glycerol-3-P in GlpT-deficient strains or glucose-6-P (and other hexose phosphates) in Uhp-deficient strains [44]. However, in *P. aeruginosa* fosfomycin can only enter cells via GlpT. As a result, *glpT* is the key target gene whose inactivation confers antibiotic resistance in *E. coli* and *P. aeruginosa* [44, 66]. In *L. monocytogenes*, due to lack of antibiotic transport the bacterium is intrinsically resistant to fosfomycin. Nevertheless, the *in vivo* virulence factor Hpt, a glucose-6-P permease, mediates the uptake of fosfomycin, conferring antibiotic susceptibility during infection [44]. The *in vitro* researches on interaction between GlpT and fosfomycin in proteoliposomes show that fosfomycin competes for the substrate-binding site of the permease and is transported by the protein. The interaction of GlpR (the GlpT repressor) with glycerol-3-P reduces its affinity for the *glpT* operator and activates GlpT synthesis [44, 67, 68].

Inactivation of the cAMP receptor protein (CRP) impairs the expression of both transporter systems and increases resistance to fosfomycin [69]. The *GlpTQ* operon contains several cAMP–CRP binding sites in a DNA stretch, negatively and positively controlled by GlpR and cAMP–CRP, respectively [70].

Modification of the antibiotic target MurA

One of the most common mechanisms resulting in Fosfomycin resistance is modification of the antibiotic MurA (UDP-N-acetylglucosamine enolpyruvyl transferase) target, which inactivates the enzyme by irreversibly binding to the protein. In *E. coli*, mutation of the fosfomycin-binding site in *MurA*, Cys115, results in resistance to this antibiotic [71]. MurA shows enzymatic activity that is susceptible to blockage by fosfomycin in a dose-dependent manner. In certain pathogenic bacteria, such as *Mycobacterium tuberculosis*, *Chlamydia trachomatis*, *Vibrio fischeri* and *Borrelia burgdorferi*, the Asp residue is present in the catalytic site of MurA proteins. Mutations in the Asp, Cys115 to Glu allow

MurA to act as intrinsic resistance to fosfomycin [54, 72–74]. However, mutations in the *murA* gene seem to be rare in clinical isolates. Mutations in the MurA sequence of *E. coli* in the clinical isolates Leu370 to Ile and Asp369 to Asn have recently been reported to represent the onset of *in vivo* Fosfomycin resistance [75]. According to another study, the identification of a salvage pathway producing peptidoglycan in *Pseudomonas putida* from UDP-MurNac is catalysed by MurA, which is the first peptidoglycan precursor [76].

Antibiotic modification

Several enzymes that inactivate fosfomycin by covalent modification cleave the carbon–oxygen bond of the epoxide moiety, including the phosphonate kinases FomA and FomB and the thiol transferases, glutathione-fosfomycin (FosA), L-cysteine-fosfomycin (FosB), ATP-fosfomycin (FosC) and water-fosfomycin (FosX), which catalyse the addition of glutathione or cysteine to fosfomycin [58, 77–80]. The loci of *fomA* and *fomB* have been identified in those strains with high-level resistance to fosfomycin and which encode polypeptides with low-level sequence identity to eukaryotic protein kinases [78]. Biochemical studies with recombinant enzymes show that FomA and FomB catalyse the phosphorylation of fosfomycin to fosfomycin monophosphate, and subsequently to fosfomycin diphosphate [61].

According to biochemical and structural studies, FosA types are metalloenzymes that catalyse the nucleophilic addition of the tri-peptide glutathione to the C1 position of the antibiotic, cleaving the epoxide ring and rendering it ineffective as an antibacterial drug [61]. FosA is a Mn (II)- and K⁺-dependent glutathione transferase. Among glutathione transferase (FosA type) enzymes found to be plasmid-borne are FosA3, FosA4, FosA5 and FosC2 [80]. FosA3 is the most prevalent gene variant, distributed mainly among both clinical and non-clinical *E. coli* isolates from Asian countries (China, South Korea and Japan) and, recently, in Europe [81–85]. FosA is encoded by clinically relevant Gram-negative species and contributes to intrinsic fosfomycin resistance. Chromosomal *fosA* genes conferred high-level fosfomycin resistance when expressed in *E. coli*, and deletion of chromosomal *fosA* in *Serratia marcescens* eliminated fosfomycin resistance [86]. However, other Gram-negative species, like *E. coli*, exhibited lower susceptibility to fosfomycin [87]. For example, clinical strains of *K. pneumoniae* producing KPC-type carbapenemase have a MIC_{50/90} of 16/64 µg ml^{−1} [88].

FosB (the amino acid sequence is 48 % identical to that of FosA) is an Mg²⁺-dependent L-cysteine thiol transferase. FosX is a Mn (II)-dependent fosfomycin-specific epoxide hydrolase and is found in *L. monocytogenes*, *Clostridium botulinum* and *Brucella melitensis* [5, 89]. In fact, FosB is produced by Gram-positive bacteria but FosA and FosX enzymes are produced by Gram-negative bacteria [45]. Nevertheless, they differ in terms of chemical mechanism, using different substrates to add chemical groups to the antibiotic. An analysis of plasmid-encoded fosfomycin resistance in

pathogenic bacteria showed a relatively low percentage of *fosA* and *fosB* genes among fosfomycin-resistant strains. Multidrug resistance plasmids encoding *fosA3*, *fosC2*, *bla_{TEM-1b}*, *bla_{CTX-M-65}* and *rmtB* carry the genes conferring resistance to fosfomycin, penicillins, cephalosporins and aminoglycosides, respectively, emerging among ESBL *E. coli* and *K. pneumonia* isolates in Asia (China, Japan and South Korea) [5]. However, little is yet known about the molecular mechanisms of fosfomycin resistance in clinical bacterial isolates. Therefore, a more in-depth knowledge of the molecular mechanisms involved in fosfomycin resistance in clinical strains could improve the efficacy of fosfomycin in the treatment of bacterial infections. Development of fosfomycin resistance may also confer a biological cost, which could be attributed to the loss of important cellular functions (decreased growth rate *in vitro* and/or *in vivo* as well as decreased virulence)[90]. Biological cost can often be reduced by compensatory mutations, and such genetic compensation has been described for several antibiotics and bacterial species both *in vitro* and *in vivo*[62].

PREVALENCE OF FOSFOMYCIN RESISTANCE

The history of fosfomycin resistance emergence goes back to 1977, when resistant *E. coli* and *Salmonella typhimurium* colonies became apparent in a zone of inhibition around fosfomycin [91]. Since then, the prevalence of resistance has varied among countries, as well as among bacterial species found in individual countries. The mechanism of resistance also plays an important role in the spread of resistance within a region. To date, not all countries have reported data on fosfomycin resistance; for certain countries, limited data are available (Fig. 3). Moreover, it should be noted that the results of fosfomycin susceptibility testing deserve careful interpretation because, in routine testing, media are not supplemented with glucose-6-phosphate, resulting in some sensitive strains appearing resistant.

Asia

Studies demonstrate that the overall fosfomycin resistance in China is higher than in other parts of the world in both human and animal hosts, which could be attributed to the mechanism of resistance. There is preliminary evidence that *fosA3* is the main mechanism responsible for fosfomycin resistance in *E. coli* isolates in China [92]. Studies from Hong Kong indicated that levels of human and animal hosts carrying the *fosA3* gene in *E. coli* isolates are 44 and 96 %, respectively [82, 93]. Although the overall rate of resistance is less than for other antimicrobial agents, increasing resistance is the main issue of consideration and concern. In a study conducted on *E. coli* isolates obtained from 20 tertiary Chinese hospitals in 2009–2010, the rate of fosfomycin resistance was 7.8 % [94]; CAO *et al.* reported a 10 % level of resistance in urine-derived *E. coli* isolates in 2010–2014 [92]. This increase in rate could be explained by the fact that high prevalence of the *fosA3* gene is concurrent with the high transferability of *fosA3*-harbouring plasmids, accounting for further transmission of resistance [92].

The prevalence of bacterial resistance other than in *E. coli* varies greatly among bacterial species. The resistance rate in *K. pneumoniae* is higher, about 60.8 % in KPC-producers and 12.5 % among ESBL-producers [95]. In addition, data have shown that *fosA3* was responsible for resistance in more than 50 % of cases [95]. As more than 71 % of *fosA3*-positive cases belonged to clonal group I, it could be inferred that high-level resistance was likely due to clonal dissemination, indicating the significant impact of this clone in Chinese hospitals [95]. On the other hand, the emergence of *fosA3* and *bla_{KPC-2}* on the same transposon in China is an issue of concern in regard to multidrug-resistant *Enterobacteriaceae* [96]. Dissemination of such resistance by a single mobilizable element threatens two classes of last-line antimicrobials in this region [96]. Besides, a drastic increase in resistance was observed in MRSA isolates. In 2010, 29.5 % of MRSA isolates were fosfomycin-resistant, reaching 70 % in 2014 [97]. Such an increase necessitates care in prescribing fosfomycin for *S. aureus*, particularly for MRSA infections in Shanghai hospitals.

The other issue is the widespread occurrence of fosfomycin resistance among animals in China, which is considerably higher than that of other countries. In a previous study, 10.2 % of *E. coli* isolates obtained from domestic pets showed resistance to fosfomycin, even though these had not received fosfomycin treatment [84]. Remarkably, molecular typing results showed that some strains isolated from pet owners were identical to those from their animals [98]. This finding tends to confirm the hypothesis that since pets are in close contact with humans, the former can act as vectors and accelerate further human-to-pet or pet-to-human *fosA3* dissemination [98]. On the other hand, in addition to *E. coli*, the *fosA3* gene has been identified in *Proteus mirabilis*, *E. fergusonii* and *Citrobacter freundii* isolates, implying the broad host range of this gene in animals in China [98]. Another study investigated the prevalence of *fosA3*-mediated resistance among *E. coli* isolates obtained from chickens in China [99]. The results revealed a not insignificant resistance rate (27.4 %), suggesting widespread distribution of *fosA3* in chickens on Chinese farms [99].

On the other hand, plasmid-mediated resistance genes other than *fosA3* have proved to be a significant challenge regarding the effectiveness of fosfomycin in China. The increase in resistance conferred by *fosB* and *fosB3* among vancomycin-resistant *Enterococci* (VRE), particularly *E. faecium*, suggests the prudent use of Fosfomycin treatment for VRE infections as well as continuous monitoring of resistance, at least in some areas of China [100, 101]. *FosX^{CC}*-mediated resistance in *Campylobacter* spp. and *fosB*-associated resistance in MRSA isolates demonstrate the diversity of plasmid-mediated resistance in China [97, 102].

Fosfomycin was approved by Japan in 1980; nevertheless, the literature shows no significant concern about resistance in this country. Despite the increasing prevalence of resistance in some areas, only 3.6 % of CTX-M-producing *E. coli* isolated from Japanese clinical settings were recognized as

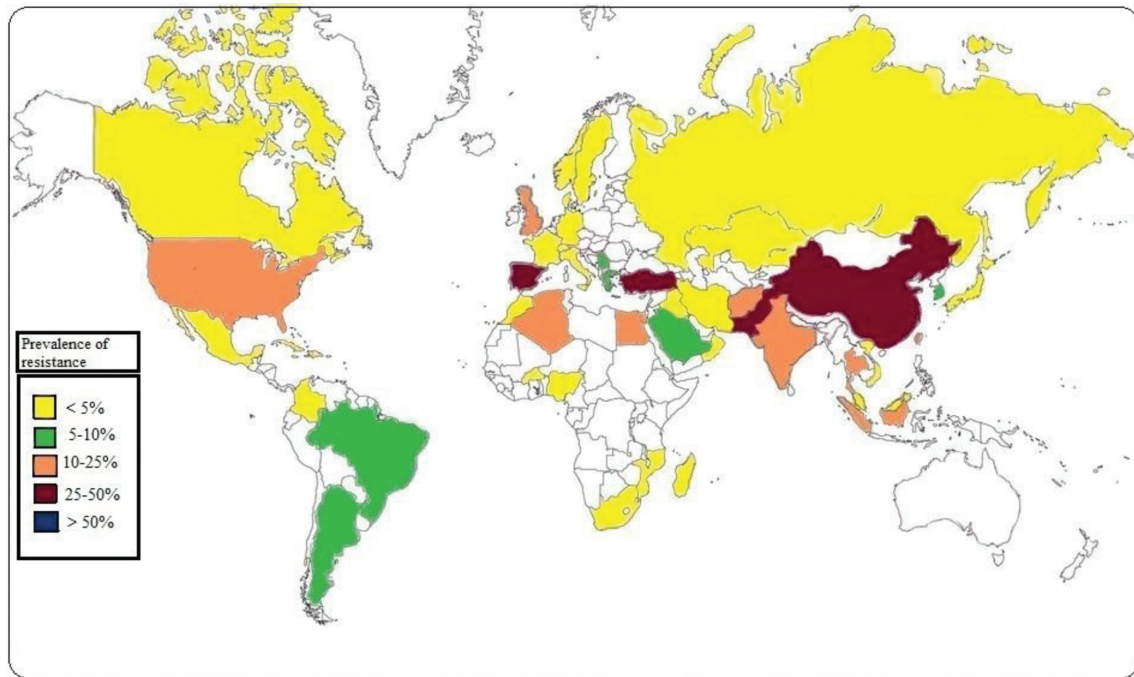


Fig. 3. The universal prevalence of fosfomycin resistance based on available data

resistant, in which *fosA3* and *fosC2* were resistance determinants [103]. However, fosfomycin resistance has drawn attention in bacterial species other than *E. coli*. *Pseudomonas aeruginosa* and Shiga-like toxin-producing *E. coli* obtained from clinical isolates showed a high resistance profile for fosfomycin [104, 105]. The most alarming finding is a novel *fosK* gene in *Acinetobacter soli* HK001 that resulted in high-level resistance ($\text{MIC} > 8000 \mu\text{g ml}^{-1}$) [106]. Studies have clarified that healthy individuals and veterinary settings also contribute in the distribution of fosfomycin resistance, as well as clinical isolates, in Japan [83]. Sato *et al.* demonstrated that 5.8 % of CTX-M-producing *E. coli* from healthy individuals were fosfomycin resistant [83]. A recent study investigated the fosfomycin susceptibility of *P. aeruginosa* isolates collected from cats and dogs in primary veterinary hospitals [107]. According to this report, 3.5 % of isolates displayed resistance to fosfomycin [107]. As mentioned previously, animals with a fosfomycin-resistant profile can be involved in the horizontal dissemination of resistance.

There are relatively few reports demonstrating the prevalence of resistance in Korea. In one study, resistance was found in 4.2 % of *E. coli* and 5.5 to 38.3 % of *K. pneumoniae* isolates, and both strains were ESBL-producers [81]. The prevalence of *fosA3* among *E. coli* isolates was higher than for *K. pneumoniae* (62.5 vs 15.4 %) [81, 108]. Based on this finding, fosfomycin is apparently not an appropriate alternative treatment for UTIs related to ESBL-producing *Klebsiella* spp. in Korea [108]. In another Korean study, the

susceptibility of 307 ciprofloxacin-resistant and/or ESBL-producing *E. coli* isolates was evaluated [109]. Only one isolate showed resistance to fosfomycin, suggesting the desirable efficacy of the drug on ciprofloxacin-resistant *E. coli* isolates [109].

Published data informing fosfomycin resistance in Iran is limited; only a few studies provide some insight into the resistance rate in this region. In more recent studies, resistance was found in 1.1 % of *E. coli* and 3.6 % of *Klebsiella* spp., but all *P. mirabilis*, *Proteus vulgaris*, *C. freundii*, *E. faecalis* and *Klebsiella oxytoca* isolates were fully susceptible [110–112]. Notably, all *Morganella morganii* isolates were 100 % resistant [110]. Mindful of the limited number of studies, the available data show that the resistance rate in Iran is lower than in other Asian countries.

Data from studies in Turkey show higher resistance rates than in an adjacent country, Iran. Although the drug has become available for clinical use only recently, the resistance rate among *E. coli* isolates ranges from 0 to 15 % in some regions [113–115]. Moreover, *K. pneumoniae* isolates showed more resistance, particularly ESBL-producing ones (26.6 %) [116]. In a 4-year study by Demir *et al.*, 4.4 % of *Enterobacter* spp. and 9.4 % of *Proteus* spp. were resistant to fosfomycin [116]. Remarkably, resistance rates were higher in *A. baumannii* and *Pseudomonas* spp., 48.6 and 56 %, respectively [116]. Similar to the data from Iran, all *M. morganii* isolates were resistant. However, the drug was found to be effective against *Serratia* and *Citrobacter* spp. and all

isolates were susceptible [116]. It should be noted that due to limited numbers of some strains, these results should be interpreted with caution [116].

A range of recent studies in Taiwan has provided evidence that fosfomycin still remains the drug of choice in the treatment of commonly encountered pathogens. The drug shows superior activity against *S. aureus*, including most MRSA strains, although resistant strains have been noted ($\text{MIC} > 512 \text{ mg l}^{-1}$) [27]. In addition, it appears to have a useful effect against both vancomycin-resistant and -sensitive *Enterococci* [27]. The drug is found to be the most active oral agent against MDR Enterobacteriaceae, with 94.5 % susceptibility [117]. Studies indicate that fosfomycin retains its activity against ESBL-producing *E. coli* isolates, with a resistance range of 0 to 4.5 % over the years [27, 118]. Besides, one study analysed decade-long data from a nationwide surveillance programme and reported a low increase in the resistance spectrum of fosfomycin in *E. coli* from community settings [119]. Non-susceptibility to fosfomycin is different in human and animal *E. coli* isolates, with relatively high resistance in pig-derived isolates (22 %) [120]. In contrast to *E. coli*, resistance in ESBL-producing *K. pneumoniae* is higher in Taiwan than in other Asian countries [121]. ESBL-producing *K. pneumoniae* isolates have shown 27.8 and 57.6 % resistance in southern and northern Taiwan, respectively [118, 121]. Moreover, the molecular mechanism responsible for fosfomycin resistance in Taiwan differs from that in other Asian countries. In Taiwan, the main resistance mechanism is amino acid variation in the GlpT and UhpT transporters, regulatory genes (*uhpA* and *pstI*) and modification of the target gene (*murA*), while, as mentioned previously, *fosA3* is the major factor in China, Japan and Korea [121].

The susceptibility pattern of fosfomycin in Pakistan is relatively similar to that in other parts of Asia. To date the drug has provided significant results against ESBL-producing and non-ESBL-producing *E. coli*, with respective non-susceptibility rates of 0–15 % [122–124]. Although resistance rates are still low, the steep rise since 2005, particularly in pus isolates, indicates an emerging threat [122]. The main concern is *Proteus* spp. which is known to be the most resistant pathogen (67 % non-susceptibility), followed by *Acinetobacter* and *K. pneumoniae* with 50 and 40 % resistance, respectively [125–127]. *Enterococcus* spp. also have shown resistance, at 40 % [128].

Due to a lack of published evidence in Afghanistan, Iraq, Malaysia and United Arab Emirates, the exact prevalence of resistance and associated molecular mechanisms remains unclear. Although no single study can demonstrate the microbial resistance rate of a country, it can help estimate the ongoing situation. In Afghanistan, Tariq *et al.* reported the fosfomycin susceptibility profile of blood culture isolates as follows: 21.7 % of Gram-negative bacilli were fosfomycin resistant, as well as 27.2 % of Gram-positive cocci [129]. In a study from Iraq, fosfomycin resistance was also lower than for other antimicrobial agents including cefepime,

cefotaxime and tetracycline in ESBL-producing *E. coli* isolates (3.1 vs 100 %) [130].

Based on the available data regarding uropathogens, fosfomycin susceptibility is promising in Malaysia, presenting only 1 % resistance [131]. On the other hand, it seems that among animals in Malaysia, fosfomycin resistance is of particular concern. The antibiotic resistance of bacterial species in seafood animals could cause massive economic losses to aquaculture operations. Moreover, resistance could be transmitted directly to humans through the handling of seafood animals with a resistant profile. To prevent such an occurrence, studies have been conducted to evaluate the susceptibility of related bacteria to a variety of antimicrobials. As a result, fosfomycin resistance has been observed in *Salmonella* spp. obtained from chicken and in *Vibrio alginolyticus* isolated from white-leg shrimps [132, 133].

Compared with other Asian reports, encouraging statistics have been reported from United Arab Emirates. Although only a single study was available, fosfomycin was reported to be active against common ESBL-producing uropathogens, among which 100 % of isolates were susceptible [134].

Fosfomycin has been available in Thailand for many years, but the resistance rate is rather low. It has been shown to be an active antibiotic against ESBL-positive *E. coli* and *K. pneumoniae*, as well as *Enterococcus* spp. [135, 136]. In a 5-year study evaluating the antimicrobial susceptibility of *S. aureus*, Fosfomycin resistance was consistently low (7.7–17 %) [137], suggesting that the drug is an effective treatment option. On the other hand, the high resistance rates in *A. baumannii* (61.7 %) and *P. aeruginosa* (50 %) imply the inefficient activity of fosfomycin against these organisms in Thailand [138].

India is considered a relatively fosfomycin-naïve population, since the intravenous formulation of drug is not currently marketed and the oral form has become available only recently. Therefore, fosfomycin has emerged as an encouraging treatment option for Gram-positive isolates [139]. Overall, *Staphylococcus*, *Enterococcus* and *Streptococcus* spp. are 99–100 % susceptible, which is much higher compared to the situation in countries such as China [140, 141]. In addition, the drug has exhibited a good effect against *E. coli* urine isolates, including ESBL-producing and non-ESBL-producing, with 83–100 % of isolates classified as susceptible [141, 142]. The susceptibility of *A. baumannii*, *P. aeruginosa*, *K. pneumoniae* and *Enterobacter* spp. is lower than in *E. coli*, but still acceptable [139]. Nevertheless, one study demonstrated that the cumulative resistance rate for fosfomycin is 45.55 % among AmpC-producing Gram-negative bacilli [143]. The *in vitro* susceptibility of these bacteria to fosfomycin suggests the latter as an efficient antimicrobial option for UTI treatment, but not suitable for infections associated with AmpC-producing isolates in India.

North and South America

Until recently, fosfomycin resistance has been reported mainly from Asian countries, but resistance finally emerged

in the Americas. Although fosfomycin was endorsed by the Infectious Diseases Society of America (IDSA) in the USA in 2010, only the oral formulation is available, and with limited experience of its use [144]. Reports from the USA show variable efficacy for fosfomycin against *K. pneumoniae*. Two studies from Ohio reported good efficacy against KPC-producing *K. pneumoniae*, even the bacteria were colistin and/or tigecyclin resistant (susceptibility 92 and 93 %, respectively) [39, 88]. On the other hand, studies from New York and Boston reported higher rates of resistance: 46.4 % in *Klebsiella* spp. and 21 and 32 % in ESBL-positive *K. pneumoniae* [145, 146]. As mentioned previously, Fosfomycin is considered a promising treatment option for VRE, which is consistent with reports from the USA. Against VRE, susceptibility rates of 98.7 and 86 % have been recorded [39, 147, 148].

In one study from Minnesota, among the 120 *E. coli* isolates studied, 98–99 % were susceptible to fosfomycin [149]. Consistent with this, another study reported only 4 % resistance among *E. coli* isolates, indicating the excellent activity of fosfomycin against *E. coli* [146]. The data available from the USA only discuss resistance prevalence, with the mechanisms responsible remaining unclear. Only one report, from Pennsylvania, demonstrated the emergence of the *fosA3* gene, conferring a high level of resistance to *E. coli* in clinical isolates [150]. The structural similarities of *fosA3*-carrying plasmids introduce the idea that this multidrug resistance plasmid has emerged in the USA through the importation of food products or human travel [150].

In a study from Argentina, >94 % of *E. coli* and *K. pneumoniae* isolates were susceptible to fosfomycin; however, the development of resistance in *P. mirabilis* was higher, with 72 % susceptibility [151]. In the same study, the *in vitro* activity of fosfomycin against *Staphylococcus* spp. and *Streptococcus agalactiae* was higher than in other countries, with 97.2 % susceptibility [151].

In Mexico, the antimicrobial activity of ESBL-producing *E. coli* and *K. pneumoniae* was assessed by Morfin-Otero et al., suggesting that fosfomycin is an effective agent, with 96.9 and 94.4 % susceptibility rates, respectively [152].

Studies of susceptibility in Canada demonstrate excellent activity for fosfomycin against *E. coli* isolates. The *in vitro* activity of fosfomycin was evaluated against 868 urinary *E. coli* isolates as part of the Canadian national surveillance study CANWARD. The drug demonstrated good activity, with an overall 99.4 % susceptibility rate [153]. Moreover, ESBL- and AmpC-producing *E. coli* isolates remained highly susceptible, presenting 94–100 % susceptibility [153]. However, some studies have reported the presence of other fosfomycin resistance determinants in Canada. One study reported the discovery of *fosA2*-producing *Enterobacter cloacae* by PCR amplification and sequencing in aquatic samples taken from a salmon river in western Canada [154], while another demonstrated *fosB*

in canine methicillin-resistant *Staphylococcus pseudintermedius* isolates [155].

The activity of fosfomycin in Brazil demonstrated results similar to other countries on that continent. In general, fosfomycin proved to be effective against the majority of *E. coli*, KPC-producing *K. pneumoniae*, *S. marcescens* and VRE [156–158]. However, higher MICs were reported for *S. aureus*, *Stenotrophomonas maltophilia* and *A. baumannii* [156, 159].

Africa

The susceptibility of fosfomycin in Africa has seldom been assessed; for some countries data are very limited (Madagascar, Senegal, Algeria, Morocco, Tunisia and Egypt) and for others there are no available data. Therefore, a comprehensive decision on fosfomycin susceptibility for the whole continent has not been reached. However, the available data could help to elucidate the current situation. In African countries generally, resistance among *E. coli* isolates can be classified according to those having a higher resistance rate, such as Egypt, and those with 0–3 % resistance, including South Africa and Madagascar [160–162]. However, the overall resistance to fosfomycin in Africa is much lower than that observed in certain Asian countries. In addition, it was introduced as the antibiotic most active against *E. coli* isolates obtained from patients with UTIs [161]. With regard to *Klebsiella* spp. isolates, the overall resistance is higher than for *E. coli*, presenting up to 36 % in ESBL-producing *Klebsiella* from Egypt and up to 12.9 % resistance in other countries [163, 164]. A study from South Africa reported the emergence of *K. pneumoniae* and *Enterobacter cloacae* co-harboring NDM-1 and KPC-2 enzymes [165]. Susceptibility testing revealed that fosfomycin and colistin were the only active agents, highlighting the significance of novel treatment options [165].

Europe

Studies have found low rates of fosfomycin resistance for all European countries, despite many years of use. Recent German data on antimicrobial fosfomycin susceptibility demonstrate its high *in vitro* efficacy. A recent multi-centre study points to low levels of resistance (no more than 1.2 %) among *E. coli* isolates [166], although 4.5 % resistance has also been reported [167]. Other German studies show susceptibility in a considerable proportion of ESBL-producing *E. coli*, *P. mirabilis*, VRE and even carbapenemase-producing *Enterobacteriaceae* against fosfomycin [167–169]. French results from the international ARES (Antimicrobial Resistance Epidemiological Survey on Cystitis) study reported high susceptibility of *E. coli* isolates to fosfomycin [170]. Moreover, other French studies show that fosfomycin remains active against ESBL-producing *E. coli* and coagulase-negative *Staphylococci*, but less active against *K. pneumoniae* [171, 172]. In Spain, fosfomycin resistance in *E. coli* shows some variation over time. Despite relatively low resistance levels, two multi-centre studies describe a large increase in resistance since 2003, in both ESBL-producing

and non-ESBL-producing *E. coli* [173, 174]. It is assumed that the increased rate in Spain is mainly due to elevated administration of fosfomycin, alongside the acquisition of resistance by CTX-M-15-producing *E. coli* isolates [174]. Although lower rates were reported for *E. coli*, resistance in ESBL-positive *K. pneumoniae* is higher (54 % nonsusceptibility), similar to findings from Taiwan [175]. Data from an ECO.SENS study, as well as from other studies, clearly show the high efficacy of fosfomycin in Austria, Portugal and the Netherlands [176–178], even against carbapenemase- and ESBL-producers [179].

UK data confirm fosfomycin as a useful therapeutic option for multidrug-resistant *P. aeruginosa* in CF patients, as well as in UTIs caused by multidrug-resistant *E. coli*, including ESBL-producers [180, 181]. However, higher resistance levels (39.5 %) among carbapenem-resistant *Enterobacteriaceae* query the impact of fosfomycin against these pathogens [182]. The susceptibility pattern of fosfomycin has been widely studied in Greece. Data from three large Greek university hospitals, consistent with other studies, show fosfomycin activity against a substantial proportion of both Gram-negative and -positive pathogens. The available data demonstrate a fully susceptible profile for *E. coli*, particularly MDR and XDR strains, *Salmonella* spp. and *S. aureus*, including MRSA [51, 182, 183]. Moreover, surprising susceptibility rates have been reported in *P. mirabilis* and *S. marcescens*, with >96 and 83 % susceptibility, respectively [184]. The high susceptibility of carbapenemase- and ESBL-positive *K. pneumoniae* (90.5 %) indicates that fosfomycin is a promising treatment option for *Enterobacteriaceae* in Greece, regardless of multidrug resistance [185]. In contrast, the drug appears to be inactive against *A. baumannii*, since in one study all 73 *A. baumannii* isolates were resistant [51] and in another study only 9 % susceptibility was reported [184].

In Switzerland and Italy, fosfomycin has been found to be the most active agent against ESBL-producing *E. coli*, presenting 100 and 98 % efficacy, respectively [186–188]. Fosfomycin susceptibility rates in Sweden are high in *E. coli*, particularly in ESBL-producing strains (97–99 %), supporting the replacement of antibiotics exhibiting reduced activity by fosfomycin [189, 190].

CONCLUSION

The available data show that fosfomycin has a high level of antimicrobial activity against a broad spectrum of Gram-positive and -negative bacteria. Although clinical evidence is still limited, fosfomycin may represent a valuable treatment option for community-acquired UTIs caused by these pathogens. Oral fosfomycin is used for the treatment of UTIs, mainly those caused by *E. coli* and *E. faecalis*. The intravenous administration of fosfomycin, which is associated with a low incidence of adverse effects, has been utilized in combination with other antibiotics for the treatment of MDR Gram-positive and -negative bacteria. In particular, data regarding the use of fosfomycin use for the treatment of such infections are awaited to further delineate

issues concerning the optimal clinical use of this antimicrobial agent.

Funding information

The authors received no specific grant from any funding agency.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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